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The nervous system and chaetotaxy of the cercaria of *Opisthioglyphe ranae* (Frölich, 1791) (Trematoda, Plagiorchiidae)

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Abstract. — The nervous system of the cercaria of *Opisthioglyphe ranae* (Frölich, 1791) is described on the basis of the distribution of acetyl-cholinesterase in the nervous tissue. Some details of the structure of the nervous system are described such as the innervation of the anterior end of the body and of the genital system. The central nervous system is found to form a full ring with the dorsal as well as the ventral commissures joining both ganglia. The sensory pattern is related to the nervous system by superimposing the distribution of sensillae on the scheme of the nerve trunks and commissures. The chaetotaxy of *O. ranae* cercaria is compared with other representatives of the genus.

Key-words. — *Opisthioglyphe ranae*, cercaria, nervous system, chaetotaxy.

Résumé. — *Le système nerveux et la chétotaxie de la cercaire d'*Opisthioglyphe ranae *(Frölich, 1791) (Trematoda, Plagiorchiidae).* — Le système nerveux de la cercaire d'*Opisthioglyphe ranae* (Frölich, 1791) est étudié sur la base de la distribution de l'acétylo-cholinestérase dans le tissu nerveux. Quelques détails de la structure du système nerveux sont décrits, tels que l'innervation de l'extrémité antérieure du corps et de l'appareil génital. Le système nerveux central forme un anneau complet avec deux commissures, ventrale et dorsale, joignant les deux ganglions. La formule sensorielle est établie en superposant la disposition des sensilles aux schémas des commissures et troncs nerveux. La chétotaxie de la cercaire d'*O. ranae* est comparée avec celle d'autres représentants de ce genre.

Mots-clés. — *Opistlioglyphes ranae*, cercaire, système nerveux, chétotaxie.

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INTRODUCTION

The nervous system in plagiorchiid trematodes was studied in some adults by LOOSS (1894) who gave a schematic picture of the cerebral ganglia, trunks and commissures. More details were observed by ZAILER (1914) in adult *Pneumonoeces* sp. Use of histochemical methods permits the visualisation of the structure of the nervous system in the cercariae (LE FLORE, 1979; GRABDA-KAZUBSKA and MOCZOŃ, 1981). The present study concerning the cercaria of *Opisthioglyphe ranae* (Frölich, 1791) is a successive step in the recognition of the structure of the nervous system in this group of trematodes. An attempt has been made also to link sensory endings on the body surface with particular nerves. The precise pattern of chaetotaxy is described. The cercaria of *O. ranae* has been described on several occasions; its

chaetotaxy was given by DOBROVOL'SKIJ (1965), NEZVALOVA (1970), DIMITROV *et al.* (1989) and RICHARD (1971) (Cercaria 4) but the descriptions differ in many respects.

MATERIAL AND METHODS

The cercariae were collected from naturally infected snails *Lymnaea stagnalis (*L.*), Galba palustris* (O. F. Müll.) and *Radix pereger* (O. F. Müll.) (= *R. ovata* Drap.) from various ponds in the environs of Warszawa. Their determination was based on morphology, body dimensions and behaviour (GRABDA-KAZUBSKA, 1969).

The nervous system was revealed due to acetyl-cholinesterase activity detected by the method of KOELLE (1951), described earlier (GRABDA-KAZUBSKA and MOCZOŃ, 1981). After the biochemical procedure the cercariae were washed in distilled water, dehydrated in graded series of ethyl alcohol, cleared in creosote and mounted on slides in Canada balsam. A very clear picture of nerve fibres was found in the cercariae several months later.

The sensory endings were detected using a simple method of staining with 2 % $AgNO_3$. After staining and washing in distilled water the cercariae were mounted on slides in Faure fluid. All drawings were made with the use of a camera lucida, for photographs Orwo NP 15 film was used.

RESULTS

THE NERVOUS SYSTEM

The nervous system was revealed due to fine crystals deposited in sites of acetyl-cholinesterase activity in the nerve cells and fibres throughout the body of the cercaria giving a clear picture of the structure of the whole system except for some details concerning the innervation of the pharynx, tail and sensory endings on the body surface.

The nervous system of the cercaria (figs 1-2) is composed of two cerebral ganglia joined by the central commissure and 6 pairs of longitudinal trunks running under the tegument to the anterior and posterior ends of the body. The trunks are connected by 8 transverse commissures. An additional pair of anterior nerve trunks runs near the median line and innervates the inner side of the oral sucker; an opposite posterior pair was not detected. The cerebral ganglia are situated laterally to and between the oral sucker and the pharynx; they are joined by a thick dorsal segment of the commissure and a more weakly developed ventral segment forming a postero-ventral loop surrounding the pharynx near its posterior border. The main trunks arise from the outer parts of the ganglia while those innervating the inner part of the oral sucker extend from the ganglia near the mid-line.

The anterior nerve trunks, ventral, lateral and dorsal, are equally well developed while in the remaining body the ventral trunks dominate. These trunks form a prominent loop on the ventral side together with the ventral segment of the last commissure. The corresponding pairs of the anterior and the posterior trunks are joined by connectives extending from the second cephalic to the first corporal commissure. Each of the anterior trunks divides into two arms :

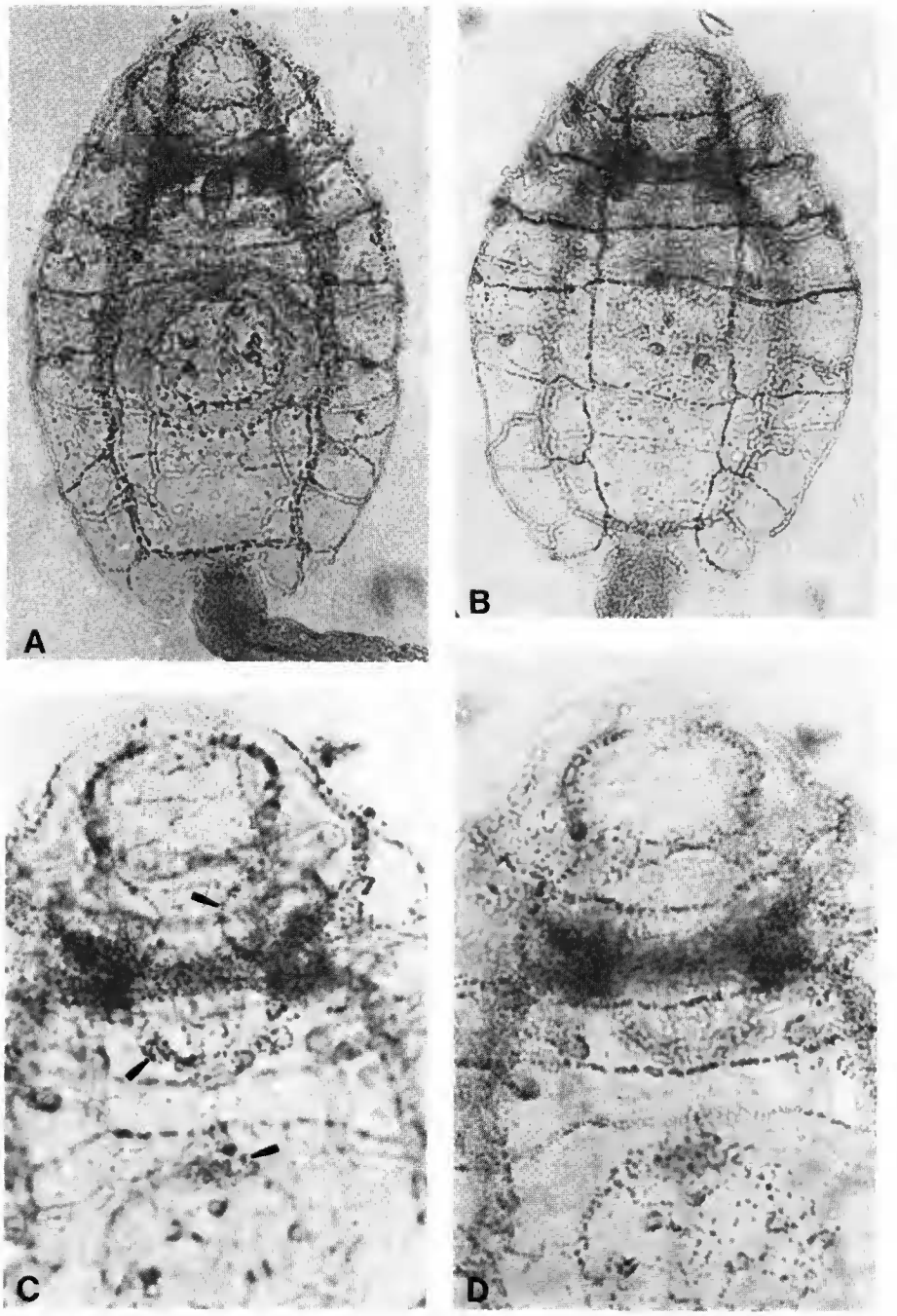

FIG. 1. — Nervous system of *Opisthioglyphe ranae* cercaria revealed due to AChE activity : A, ventral view; B, dorsal view; C, innervation of oral sucker, cerebral ganglia with commissures, genital ganglion (arrows); D, innervation of the anterior end — cephalic and preacetabular commissures.

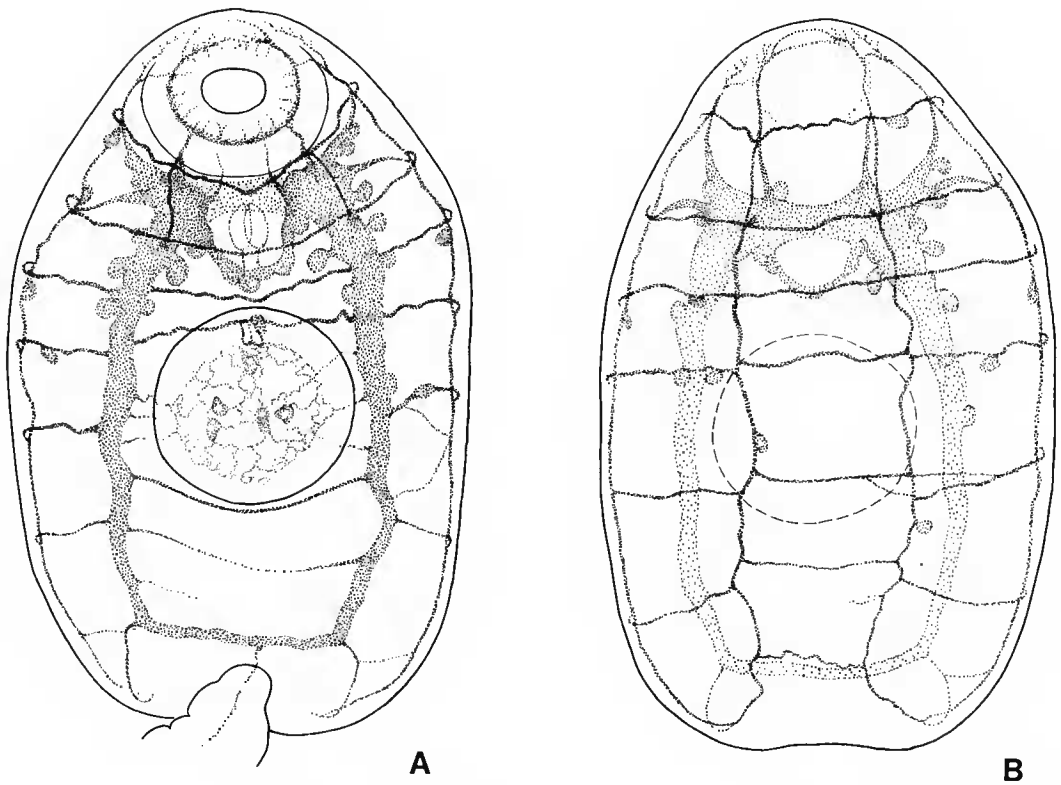

FIG. 2. — Nervous system of *Opisthioglyphe ranae* cercaria : A, ventral; B, dorsal view.

an inner and outer one at about one third of length from anterior end. The inner arm joins directly with the first cephalic commissure forming the oral ring, while the outer arm splits into numerous fibres running to the body surface where they probably terminate in sensory cells.

In the cephalic part of the cercaria two commissures are present — the first forming the oral ring and the second situated in midway of the anterior trunks. Anterior to the oral ring, thin fibres originating from the dorsal trunks surround the stylet. Posterior to the ganglia and before the ventral sucker three more pronounced commissures form well marked transverse rings encircling the whole body, equally distant from one another. The postacetabular three commissures form more distant, less pronounced and irregular rings, sometimes with segments shifted or lacking, as well as with additional fibres especially between the ventral trunks. The caudal nerve fibre arises from the ventral segments of the last commissure, at mid-line, and runs along the tail. Its passage through the tail was not visible being hidden by well developed muscles showing traces of acetyl-cholinesterase.

Except the first commissure surrounding the oral opening the oral sucker is innervated by inner trunks piercing the sucker at the bottom and splitting into thin fibres forming a loose net

lining the oral cavity. This net extends to the border of the mouth. Innervation of the pharynx is rather difficult to interprete and the pharyngeal nerve plexus is not clear. In some specimens it seems that the pharynx is supplied by nerve fibres arising from the ventral segment of the central commissure or these nerves run parallel to the commissure. The ventral sucker is supplied by the ventral trunks through thin fibres, one of them is usually a prolongation of the lateral segment of the first postacetabular commissure. These fibres form a feebly marked ring in the ventral sucker, filled by a loose net of fibres and 5 (sometimes 6) cell bodies.

Anterior to the ventral sucker, at the mid-line, there is a small nerve ring and a cell connected with the third postacetabular commissure, forming a primordium of the innervation of the genital atrium. It lies perpendicularly to the body surface, at an angle to the sagittal plane.

Chaetotaxy

The sensillae are grouped according to the system proposed by Bayssade-Dufour *et al.* (1989) for plagiorchiid cercariae : 4 transverse rings are discerned in the cephalic region (CI, CII, CIII and CIV) and 6 on the body (AI, AII, AIII, PI, PII, PIII). Moreover, in the distribution of sensillae, 7 longitudinal lines are discerned in the cephalic region (numbers : 0-6) and 3 lines on the body corresponding to ventral (V), lateral (L) and dorsal (D) trunks. Most sensillae are situated at crossings of the nerve trunks with commissures.

Cephalic region (fig. 3)

$CI = 1\ CI_2,\ 3\ CI_3,\ 3\ CI_{4,5}$
$CII = 0 - 1\ CII_0,\ 1\ CII_1,\ 1\ CII_2,\ 1\ CII_3,\ 1\ CII_4,\ 2\ CII_5$
$CIII = 1\ CIII_1,\ 1\ CIII_2,\ 2\ CIII_3,\ 10\ CIII_4,\ 15\ CIII_5$
$CIII\text{-}IV = 2 + 2 + 2\ CIII\text{-}IV_5$
$CIV = 3\ CIV_1,\ 4\ CIV_2,\ 0\text{-}1\ CIV_3,\ 3\ CIV_4,\ 4\ CIV_5$

One more sensilla occurs in dorsal and lateral rows, posterior to groups recognized as belonging to CIV ring. They are marked here as $1\ CV_4$ and $1\ CV_5$. The sensilla CIV_3 was present only in a part of the cercariae, mainly in those emerging from *Galba palustris*.

Body (fig. 4)

$AI = 2AI_V,\ 4\text{-}6AI_{L1},\ 0\text{-}2\ AI_{L2},\ 4 + 4AI_D$
$AII = 1AII_V,\ 3\text{-}4AII_L,\ 3AII_D$
$AIII = 1AIII_V,\ 1\text{-}3AIII_L$
$PI = 2PI_V,\ 6\text{-}10PI_L,\ 1PI_D$
$PII = 2\text{-}5PII_L$
$PIII = 3\text{-}6PIII_L$

Two additional sensillae marked here as AI_{L2} are present in many but not in all cercariae. They are clearly separated from the lateral group AI_{L1}. Three dorsal sensillae placed in AII ring according to Richard (1971) may be also recognized as belonging to AIII ring.

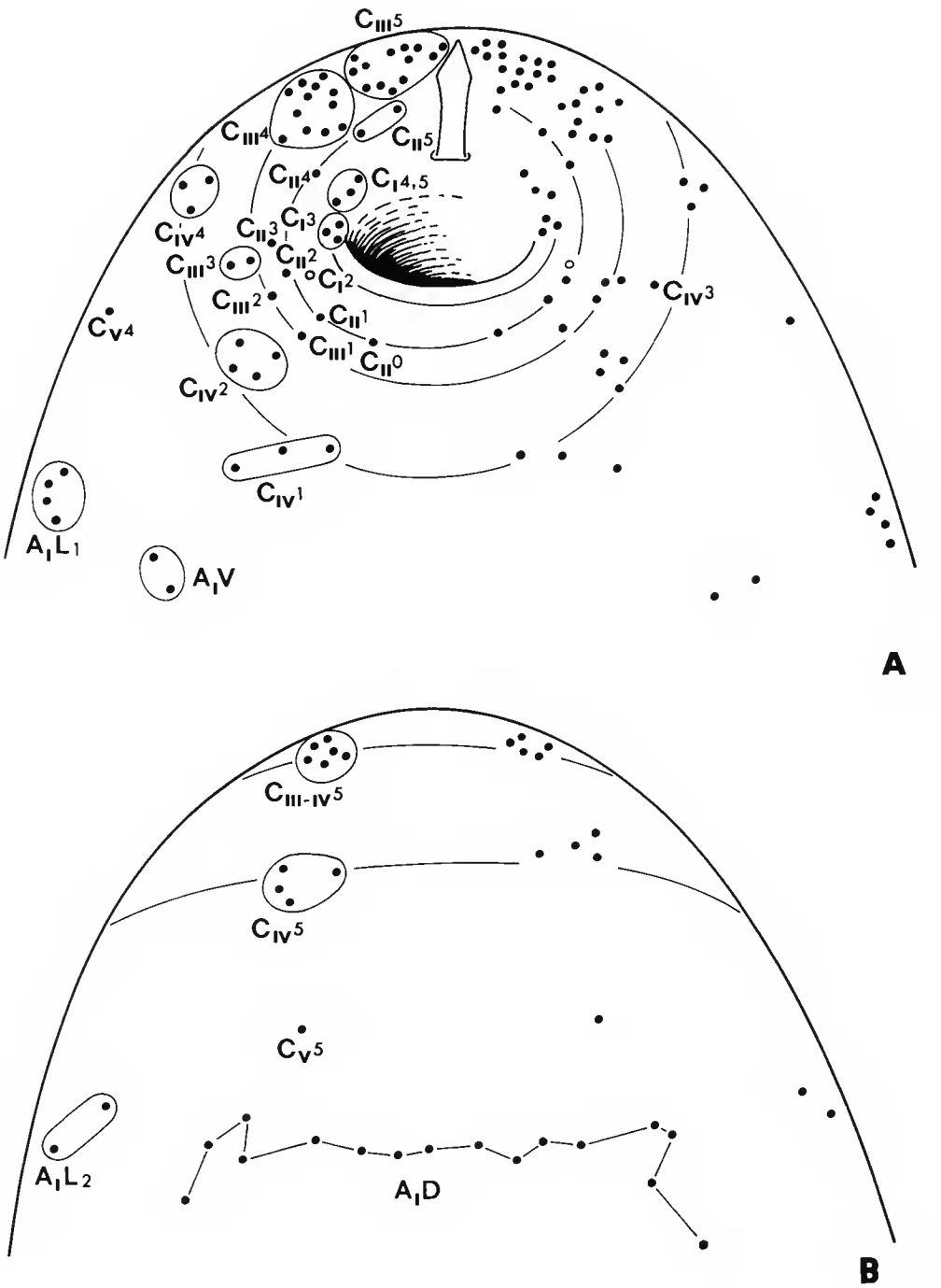

FIG. 3. — Chaetotaxy of *Opisthioglyphe ranae* cercaria, cephalic region : A, venttral; B, dorsal view.

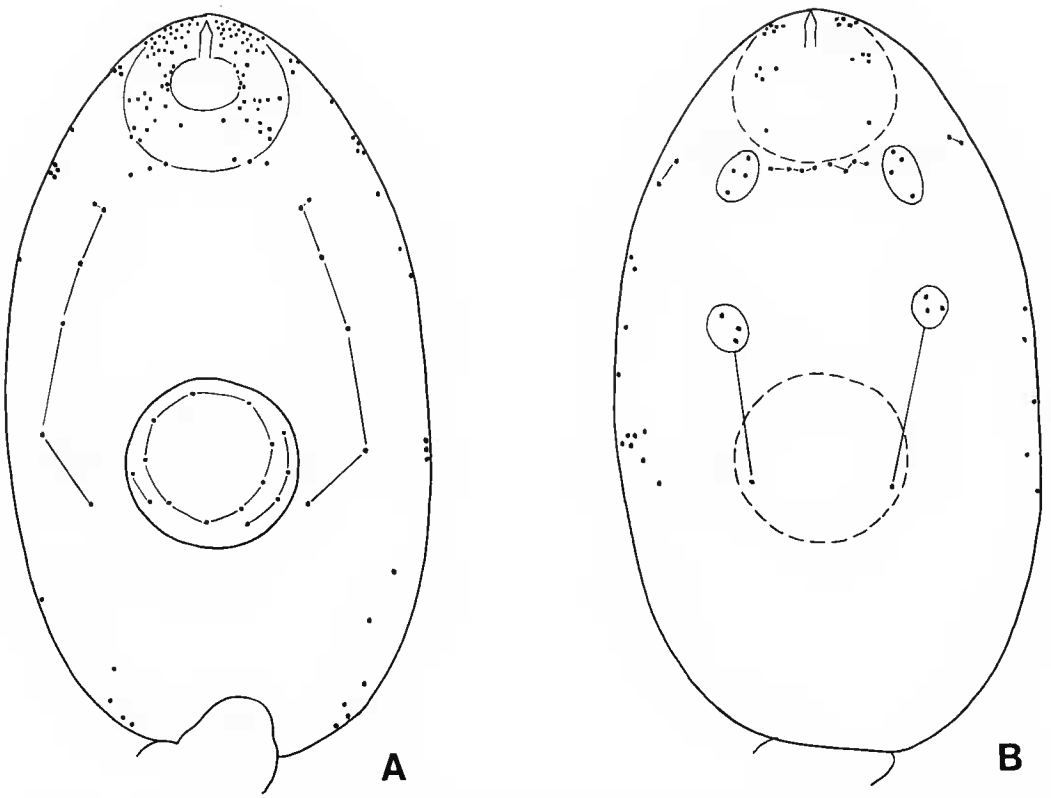

FIG. 4. — Chaetotaxy of the cercaria of *Opisthioglyphe ranae*, body : A, ventral; E, dorsal view.

The ventral sucker bears the sensillae arranged in two rings : 9SI + 0-6SII. The number of sensillae in SII ring is very variable.

The tail bears 2UD sensillae characteristic of plagiorchiids.

DISCUSSION

The nervous system of *O. ranae* cercaria as described in the present paper corresponds in general with that described by LOOSS (1894) in adult *O. ranae* (*op. cit.*, fig. 157) and in the cercaria of *Haplometra cylindracea* (Zeder, 1800) by GRABDA-KAZUBSKA and MOCZOŃ (1981). However, more clear preparations of *O. ranae* permit the correction of the earlier description.

The most important difference lies upon the fact that the cerebral ganglia are joined by the ventral as well as by the dorsal commissure forming thus a full ring around the prepharynx and the pharynx. In *H. cylindracea* the median part of the ventral segment was invisible giving

thus a picture of two short trunks, regarded as pharyngeal ones. No structures corresponding to such trunks have been recognized in *O. ranae* cercaria. A less important difference concerns the number of connectives which in *O. ranae* are 6, joining all opposite pairs of nerve trunks.

More details were also seen in the structure of the cephalic nerves, e.g. branching of the anterior trunks into inner and outer arms similar to that described by ZAILER (1914) in adult *Pneumonoeces* sp., innervation around the stylet, a nerve plexus inside the oral sucker, and innervation of the genital atrium.

Although the preparations of the nervous system of *O. ranae* cercaria were very clear, showing numerous thin fibres forming the tegumental plexus, the sensory endings on the body surface remained invisible. So, the connections between particular sensillae and corresponding parts of the nervous system were estimated by superimposing the picture of sensillae on that of the nerve trunks and commissures. The results are presented in the pattern of chaetotaxy. According to BAYSSADE-DUFOUR *et al.* (1989) four rings are discerned in the cephalic region of plagiorchiid cercariae : the first three rings being joined with the oral commissure while the fourth ring with the second commissure. However, it can not be excluded that the sensillae the CI ring, situated at the inner margin of the oral sucker, are supplied by nerve fibres originating from inner trunks. Single sensillae CV_4 and CV_5 are related to connectives.

In the remaining part of the body 6 rings of sensillae corresponding to 6 commissures are discerned as well as 6 longitudinal rows corresponding to ventral, lateral and dorsal pairs of nerve trunks. The median ring discerned by RICHARD (1971) is regarded as being represented by the first postacetabular ring PI and the corresponding commissure.

The sensory pattern of the present cercaria fits well the chaetotaxy of Cercaria 4 after RICHARD (1971), a larva thought to be *O. endoloba* (Dujardin, 1845) and the chaetotaxy of *O. ranae* after DIMITROV *et al.* (1989). Taking no notice of the different methods of the description of chaetotaxy by RICHARD (1971), DIMITROV *et al.* (1989) and the present authors, our cercaria differs from Cercaria 4 only by the presence of two sensillae in AI_{L2} group and additional sensilla in P_IV. So, we consider both these cercariae as representing the same species and we uphold the earlier opinion by GRABDA-KAZUBSKA (1967) concerning the identity of *O. ranae* and *O. endoloba*. Drawings of the chaetotaxy of *O. ranae* cercaria by DOBROVOL'SKIJ (1965) and NEZVALOVÁ (1970) are too inaccurate to be compared more precisely with our cercaria (DOBROVOL'SKIJ wrote that 8 sensillae are in the dorsal transverse row, but only 6 are marked in the drawing. The number of sensillae is also incomplete in the drawing made by NEZVALOVÁ).

In comparison with other cercariae representing the genus *Opisthioglyphe* (RICHARD, 1971; VAUCHER, 1972; BOCK, 1983) the chaetotaxy of our cercaria shows important differences : frequent lack of CII_O sensilla, doubled number of AI_D median sensillae (8 instead of 4), lack of ventral and dorsal sensillae in postacetabular PII and PIII rings.

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